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Inoculation of Legume Crops

Legumes are becoming increasingly important in Alberta. Specialty legume crops such as field beans, fababeans, peas, soybeans, and lentils have considerable potential in southern and central Alberta. Throughout the province, forage legumes have been an important constituent of hay and pasture crops for many years. Legumes have the potential of producing high yields, mainly because of their ability to fix nitrogen from the air with little or no requirement for fertilizer nitrogen. The rising cost of nitrogen fertilizer increases the value of legume crops (Table I).

Legumes form a unique symbiotic relationship with certain soil bacteria (rhizobia) through which nitrogen is made available to the plant. Atmospheric nitrogen is taken from soil air spaces and converted to a usable form in a process called **nitrogen fixation**. Gaseous nitrogen is the most abundant element in the atmosphere and is continually replenished so the supply remains constant.

Nodules

Rhizobia invade the roots of legumes, multiply and cause a swelling, forming nodules which become miniature nitrogen fertilizer factories. The shape and size of nodules varies with legume species (Figures I, 2, 3, 4). For the process to work, bacteria must be present in the soil, they must infect the root and must be able to effectively fix nitrogen. Different species of rhizobia require specific host plants for nitrogen fixation to occur. Sometimes bacteria infect the roots but are not able to fix nitrogen. In other situations bacteria able to fix nitrogen cannot enter the root and no nodulation occurs. To ensure that effective nitrogen-fixing nodules are formed, all legumes should be inoculated with the correct rhizobia using a proper method.

What is an Inoculant

An inoculant consists of one or more strains of a live bacterium (*Rhizobium* species) in a carrier material which supports and protects the bacterium. The most common carrier material is peat moss with lime added to ensure that the pH is not acidic. The peat has a very high water-holding and buffering capacity and thus protects the rhizobia against drying and unfavorable soil pH levels.

Two types of inoculant products are sold in Canada for on-the-farm use: 1) powdered inoculant - a finely-ground peat which contains over a billion rhizobia per gram and must be applied directly to the seed, and 2) granular inoculant - a small granule the size of a clover seed which contains as many rhizobia as the powdered inoculant but is designed to be applied in the furrow with the seed.

Inoculant Quality

All legume inoculants sold in Canada are registered under the Fertilizers Act administered by Agriculture Canada. Legume inoculant products are regularly sampled and tested by Agriculture Canada to ensure that they meet rigid quality standards. The inoculant package label contains the following information: crop species for inoculation; name (species) of *Rhizobium* bacterium; amount of seed the package will inoculate; federal registration number; expiry date; and recommended method(s) of application. This ensures that good nodulation and nitrogen fixation will result if the inoculant is properly stored and correctly applied. Inoculants are live products and should be kept refrigerated (or at least cool), avoiding desiccation and the harmful rays of the sun.

Inoculant Specificity

Each legume plant or group of legume plants has a unique species of *Rhizobium* which will form nodules and fix nitrogen. In fact, the rhizobia are often named after the plants which they nodulate. For example, *Rhizobium phaseoli* forms nodules only on *Phaseolus vulgaris* (field bean) and *Rhizobium trifolii* only on *Trifolium* species (white, red and alsike clover). The commercial inoculants available are prepared for specific cross-inoculation groups which are made up of one or more legume species (Table 2).

Inoculation with the proper inoculant is essential (Figure 5, 6). The inoculant package label clearly states the designated host plant.

Methods of Inoculation

Inoculation refers to the addition of effective rhizobia to legume seed prior to planting. The most common type of inoculant sold in Canada is the powdered peat-base inoculant. To be effective the

Copies of this and related publications may be obtained from the Print Media Branch, Alberta Agriculture, 7000 - 113 Street, Edmonton T6H 5T6 or Alberta Agriculture's District Offices.

Table 1. The value of annual nitrogen fixation by legumes in Brown, Black, and Gray soils in Alberta

	Brown[1]				Black[2]				Gray Luvisol[2]			
	Annual fixation		Value of N[3]		N_2 fixed		Value of N		N_2 fixed		Value of N	
	kg N/ha	lb N/ac	$/ha	$/ac	kg N/ha	lb N/ac	$/ha	$/ac	kg N/ha	lb N/ac	$/ha	$/ac
Alfalfa	300	267	210	85	86	77	60	25	221	197	155	63
Alsike clover	-	-	-	-	76	68	53	22	151	134	106	43
Bird's-foottrefoil	-	-	-	-	72	64	51	20	95	85	67	27
Red Clover	-	-	-	-	125	111	88	36	167	149	117	48
Sweet-clover	250	223	175	71	62	55	44	18	107	95	75	30
Chickpea	120	107	84	34	-	-	-	-	-	-	-	-
Fababean	300	267	210	85	132	118	92	38	-	-	-	-
Field bean	70	62	49	20	-	-	-	-	-	-	-	-
Lentil	150	134	105	43	-	-	-	-	-	-	-	-
Pea	200	178	140	57	-	-	-	-	-	-	-	-
Soybean	150	134	105	43	-	-	-	-	-	-	-	-

1 N_2 fixation determined by ^{15}N (Rennie, 1984) on irrigated soils

2 N_2 fixation determined by total-N yield difference method (Rice and Hoyt, 1980) except for fababeans determined by acetylene reduction assay (Penney, 1974)

3 Value based on cost of nitrogen fertilizer @ $0.70/kg N ($0.32/lb N)

NODULATED ROOTS OF LEGUME CROPS

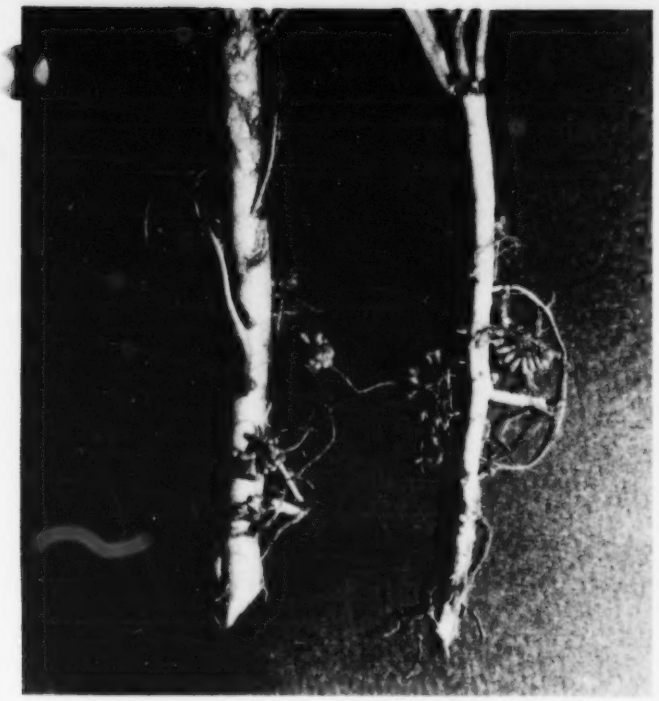

Figure 1. Alfalfa*

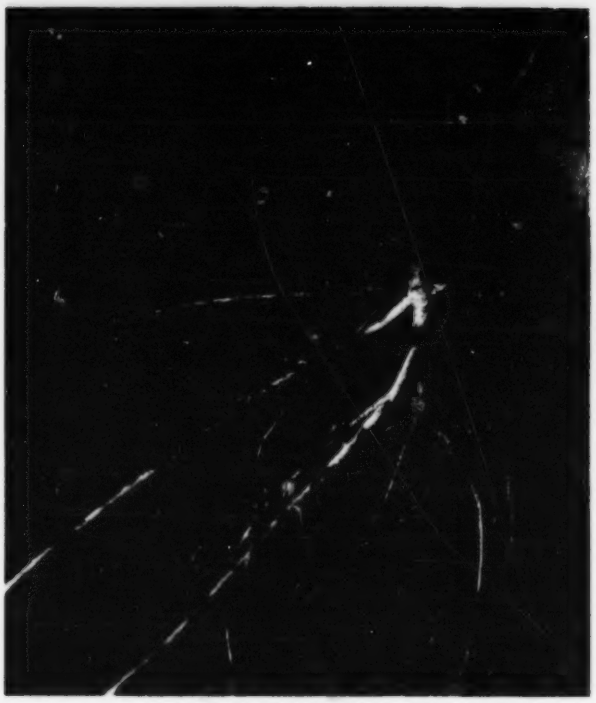

Figure 2. Alsike clover

Figure 3. Soybeans

Figure 4. Field peas

* Alfalfa nodules are often located at some distance from the main root.

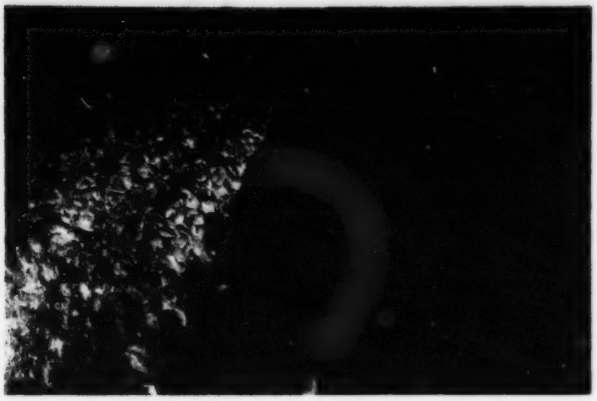

Figure 5. Soybean crop
L - uninoculated
R - inoculated

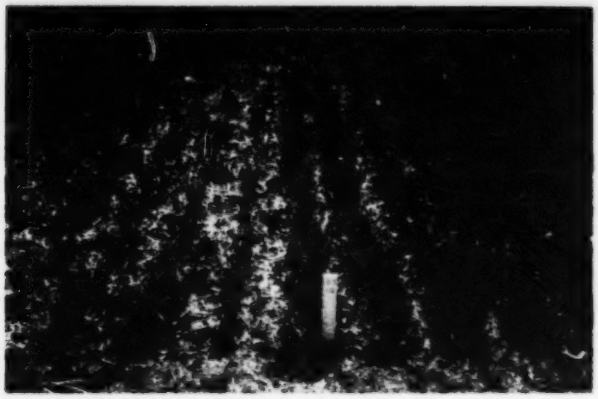

Figure 6. Alfalfa crop
L - uninoculated
R - inoculated

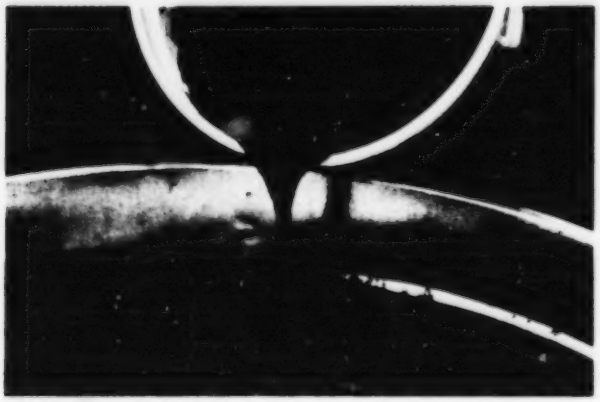

Figure 7a.

Figure 7b.

A convenient and reliable inoculation method is with one-half the recommended amount of peat-base inoculant made into a slurry with the sticker solution, the slurry mixed well with seed (7a), and then the remaining inoculant (7b) added with further thorough mixing.

Figure 8. Interior of soybean nodule showing pink color of an actively nitrogen-fixing nodule and an inactive nodule (brown color). White and green colored nodules are also inactive.

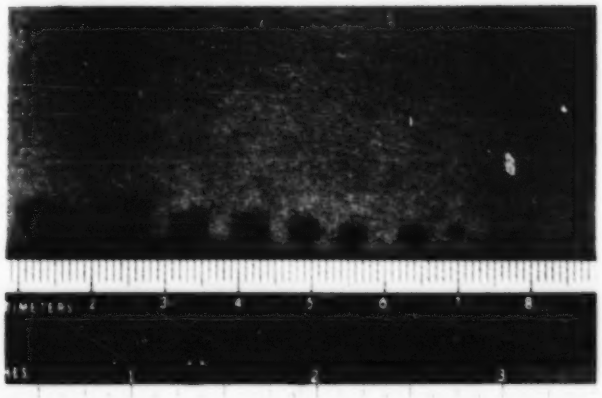

Figure 9. Different size nodules from field beans.

Table 2. Cross-inoculation groups and Rhizobium-legume associations

Cross-Inoculation Group	*Rhizobium* species	Legumes
Alfalfa	*R. meliloti*	alfalfa, sweet-clover
Clover	*R. trifolii*	white, red, alsike clover
Pea	*R. leguminosarum*	field peas, garden peas common vetch, lentils, fababeans
Bean	*R. phaseoli*	field beans, garden beans
Soybean	*R. japonicum*	soybeans
Sainfoin	*Rhizobium* spp. (special strains)	sainfoin
Bird's-foot trefoil	*R. loti*	bird's-foot trefoil
Cicer milk-vetch	*Rhizobium* spp. (special strains)	cicer milk-vetch
Crown vetch	*Rhizobium* spp. (special strains)	Crown vetch

inoculant must adhere to the seed to ensure that the rhizobia are close to the newly emerging roots (Table 3). There are three common methods recommended for applying powdered inoculants.

Water slurry method - The inoculant is suspended in water and then mixed thoroughly with the seed until each seed is coated uniformly with the inoculant powder.

Sticker solution method - This is the most effective method since it 'glues' the inoculant to the seed. The adhesive material or inoculant sticker also serves to feed the rhizobia and protects them from drying conditions on the seed. Several sticker products are available commercially from the companies that manufacture inoculants. The best non-commercial sticker is a 40 per cent (w/v) water solution of gum arabic. Ten per cent water solutions of wallpaper glue (containing no lead or mercury) or powdered milk are also very effective. Other good stickers are 10 per cent water solutions of common table sugar, corn syrup or honey. Most of these stickers require some heating to bring them into solution.

The seeds are placed in a large container (cement mixer, pails) and sufficient sticker is applied to slightly wet the seed. Avoid adding excessive sticker.

The inoculant powder is sprinkled on the seeds while mixing until the seeds are uniformly coated. An alternative is to substitutewater for the sticker solution in the water-slurry method (Figure 7). The seeds are then allowed to dry for one to two hours. They may then be planted with normal seeding equipment. Seeds treated with excess sticker and that have not been properly dried will clog the seeding equipment. This inoculating operation may be performed one to two days before the actual seeding date if the inoculated seeds can be stored in a cool dark place.

Inoculant Slurry Method - This is an effective method of inoculating large and small legume seeds. The inoculant is premixed with sticker and water and this slurry is metered onto the seed as it flows through a grain auger or drill fill. When done properly this method is as effective as the sticker soloution method and saves labor and time.

Detailed instructions are given on inoculant and sticker package labels.

Granular Inoculants

Granular inoculants are available for soybeans and field beans. The small granules are designed to be applied at low but very uniform rate in the seed furrow. The granule size is similar to that of many herbicides and is normally applied through biocide

Table 3. The effect of different methods of inoculation of alfalfa seed on forage yield (Rice 1983)

	Dry matter yield, tonne/ha (tons/ac)			
	First year		Second year	
No inoculant	2.1	(0.9)	1.7	(0.8)
Inoculant without sticker	2.1	(0.9)	1.7	(0.8)
Inoculant with sticker	3.3	(1.5)	2.2	(1.0)
Pre-inoculated in seed coating	3.4	(1.5)	2.4	(1.1)

applicators designed for accurate application at low rates. The normal application rate is 5 kg/ha (4.5 lb/ac) although higher rates are recommended in soils which have not grown the specific legume before or in problem soils. Granular inoculants have better rhizobial survival in dry soils. Also granular inoculants are easy to apply and use with fungicide-treated seeds, where the seed-applied inoculant may be injured.

Pre-inoculated seed

Pre-inoculated legume forage seed is becoming more common on the market. Pre-inoculation refers to the process of inoculating seed in bulk well in advance of the planting date. Various procedures are used such as pelleting or coating, a vacuum process that forces the rhizobia under the seed coat, and a clay-base inoculant that adheres to the seed by electrostatic forces. Pre-inoculated seed eliminates the extra time and effort required to apply inoculants on the farm, but has a major disadvantage in that the viability of rhizobia declines rapidly once applied to the seed. Also, it is difficult to store large lots of pre-inoculated seed and maintain rhizobia viability. If the viability of the rhizobia on pre-inoculated seed is maintained, adequate effective nodulation will be obtained. The quality of most pre-inoculated seed products, particularly coated seed is now sufficient for adequate nodulation.

Steps to Successful Inoculation and Nodulation

Certain management practices may influence the effectiveness of the bacteria in successfully nodulating legume crops.

- Commercial inoculant - is a living culture of bacteria and is always labelled with an expiry date. The culture is most effective when used before this date. Store inoculum in a refrigerator (do not freeze) or other cool dark places. Refrigerated inoculant maintains its viability longer.
- Farm Inoculated seed - should be sown as soon as possible. If this cannot be done the same day, place the seed in plastic bags and store in a cool dark place for two days at most. If seeding is delayed longer, reinoculate seed.
- Soil conditions - survival of rhizobia bacteria will be reduced in acid soils. Lime should be applied before seeding if soil pH is below 6.0. There are now strains available in the alfalfa group selected for their ability to survive and nodulate in soils with pH as low as 5.6.

  Survival of nodule bacteria is also affected by dry soil. Inoculated seed should be sown into a firm moist seedbed, if possible. Too much moisture can also be harmful since rhizobia need oxygen, and wet soil with reduced soil air levels may cause serious bacterial losses.
- Seed treatments - fungicide-treated seed requires special consideration. Generally, seed treatments affect nodulation less if the seed is properly inoculated with a high quality inoculant and is planted immediately following inoculation. Thiram is compatible with alfalfa rhizobia.

  Captan is particularly harmful to seed-applied rhizobia in large-seeded legumes. Increasing the inoculant application above the recommended rate will help reduce the harm done by the fungicide to the rhizobia.

  If legumes are being planted with any of the above mentioned stress conditions present it is recommended that three to five times the usual amount of inoculant be used.
- Checking for nitrogen fixation - to determine if nitrogen fixation is occurring, dig up a plant in the field, locate the nodules and crush a few of them. If nodules are red or pink inside, fixation is occurring (Figure 8). A well-nodulated plant has 20-100 nodules at flowering and nodule size may vary considerably on the same plant. (Figure 9).

Guide to Successful Nodulation

- inoculate all legume seed with the appropriate fresh inoculant
- purchase commercial inoculant specific to the legume being sown
- store inoculant in sealed containers and in a cool dark dry place until used
- use the sticker method of inoculation
- inoculate seed immediately before seeding
- sow inoculated seed into a firm moist seedbed
- careful seedbed preparation and good management practices are also important.

Inoculants are sold in Alberta at several retail outlets. These products have been tested and, if properly stored and used, will ensure legume yields equal or higher to those obtained with fertilizer nitrogen. Contact your local district agriculturist, or the research branch of Agriculture Canada for more details.

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